User Manual

Thermo Scientific Pierce G2 Fast Blotter

Version 1

May 2013

Thermo SCIENTIFIC

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SECTION 1

Overview

The Thermo Scientific™ Pierce™ G2 Fast Blotter is designed for rapid semi-dry transfer of proteins from polyacrylamide gels to nitrocellulose or PVDF membranes. Traditional Western blotting techniques require a transfer of one hour to overnight to achieve good transfer efficiency. When used in conjunction with Thermo Scientific™ Pierce™ 1-Step Transfer Buffer, the Pierce G2 Fast Blotter is designed to provide transfer efficiency in 5-10 minutes that is equivalent to, or better than, traditional blotting techniques without the need for gel pre-equilibration. This significant reduction in protein transfer time is accomplished by optimizing the ionic strength of the transfer buffer and increasing the current [amps (A)/cm^2] flowing through the transfer stack. The system has been verified to work with commonly used pre-cast and homemade SDS-PAGE gels. The Pierce G2 Fast Blotter can also be used for standard semi-dry transfer protocols with Towbin buffer.

Contacting Thermo Fisher Scientific

For technical or service assistance on the Pierce G2 Fast Blotter:

• U.S. Customers: 1-800-874-3723

Fax: 1-800-842-5007

• International Customers: 1-815-968-0747

Fax: 1-815-968-4736

Technical Support email for U.S. and international customers outside Europe:

pierce.ts@thermofisher.com

Technical Support email for European customers:

perbio.eurotech@thermofisher.com

For more information about the Thermo Scientific Protein Biology product line, please visit thermoscientific.com/pierce

Instrument Overview

Unpacking and Setup Instructions

Unpack your new Pierce G2 Fast Blotter Control Unit and/or Cassette.

Verify that all of the accessories listed below are included with your blotter package. Please note that the control unit and the cassette can be purchased separately or combined.

Your blotter purchase includes the following accessories (each quantity 1):

Included Accessories (Control unit)

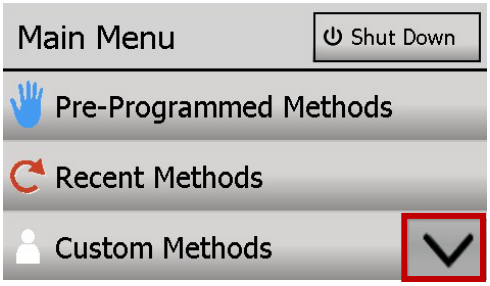
Pierce G2 Fast Blotter Control Unit
Quick Start Guide
Blot Roller
Power Cord with C13 Connector

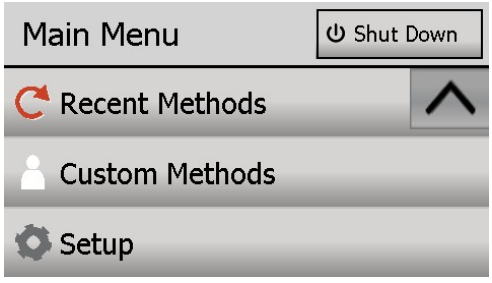
Included Accessories (Cassette)

Pierce G2 Fast Blotter Cassette

User Interface

The user interface consists of a color LCD menu touchscreen on the top of the control unit. From the **Main Menu**, five selections are available. Touching the lower right **Down** arrow allows you to see the last two methods and the **Setup** button.





1. Pre-Programmed Methods This feature allows you to quickly access the recommended amperage, voltage and time for the size and number of gels that you intend to simultaneously transfer. The blotter is only intended for use with the following consumables:

    - Pierce 1-Step Transfer Buffer
    - Bio-Rad™ Trans-Blot™ Turbo™ Transfer Stacks
    - Towbin Transfer Buffer for Standard Semi-Dry Transfer (only)

2. Recent Methods Access to recently run methods listed from most to least recent.

3. Custom Methods Create, run and save a custom transfer method.

4. Setup Control audio settings, view software and hardware versions, and install software updates to the user interface.

5. Shutdown Safely shuts down the device.

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SECTION 2

Instrument

Installation Guidelines

IMPORTANT Review and implement guidelines for proper set-up before installation. Locate the imager away from water, solvents, corrosive materials, strong magnetic fields and vibration sources.

Space Requirements

The blotter requires a stable laboratory bench or table. Provide a minimum clearance of 3 inches (7.62cm) at the rear of the blotter to allow for adequate ventilation for cooling and access to the power cord and switch. In addition, allow sufficient space at the front of the blotter for docking and removing the cassette with/from the control unit.

Table 2.1. Control unit dimensions.

Dimensions	Inches	Centimeters
Width	6.44	16.4
Depth	10.88	27.6
Height	6.06	15.40

Table 2.2. Cassette dimensions.

Dimensions	Inches	Centimeters
Width	10.13	25.7
Depth	10.94	27.8
Height	1.38	3.5

Electrical Requirements

Note A grounded circuit capable of delivering the appropriate current and voltage is required for installation. Electrical requirements can be located on the rear panel of the blotter. The blotter electrical system will adjust to the proper voltage for the respective country. Connect the power cord to the rear left side panel of the device and plug into a grounded power outlet.

WARNING After running at high current, the anode and cathode plates can become hot. Use caution when separating the gels and stacks from the plates. When continuously processing multiple samples at ~5A for a maximum of 2 hours, allow the cassette to cool for 30 minutes or use multiple cassettes to avoid excessive cassette heating.

WARNING Blotter use outside of the workflows described in this manual may put the operator at risk of dangerous exposure to electrical shock. Do not use this instrument for any purposes or in any configurations not described in this manual.

WARNING The blotter control unit can contain dangerous electricity and is not designed to be opened by the user. Disconnect all power to the control unit before maintenance by a qualified technician.

WARNING Do not overfill the cassette with liquid. Excess liquid can overflow into the control unit and possibly cause electric shock. Follow the appropriate instructions for reagent amounts and empty any remaining liquid in the cassette upon run completion.

Table 2.3. Blotter electrical ratings.

Blotter Electrical Parameter	Rating
Supply Voltage (VAC)	120-240
Frequency (Hz)	50-60
Maximum Power Rating (W)	168
Fuse (Power Center)	T4AL, 250V, 4A

Table 2.4. Blotter electrical requirements for major countries.

Region	Voltage (VAC)	Frequency (Hz)	Amps (A)
US and Canada	120	60	1.0
Europe	220	50	0.5
UK	240	50	0.5
Japan	100	50	1.2
China	220	50	0.5
India	230	50	0.5
South Korea	220	60	0.5

Environmental Requirements

For optimal performance, maintain a constant laboratory temperature range of 15-25°C (59-77°F) with relative humidity between 20-70%. Do not place blotter in direct sunlight.

Blotter Images with Feature Locations

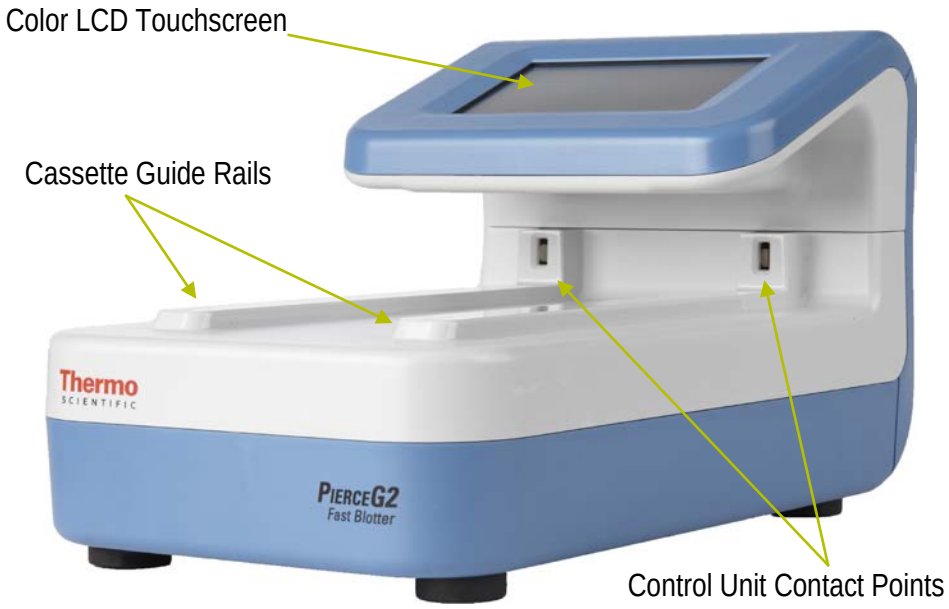


Figure 2.1. Blotter Control Unit front and side views detailing main components of control unit.

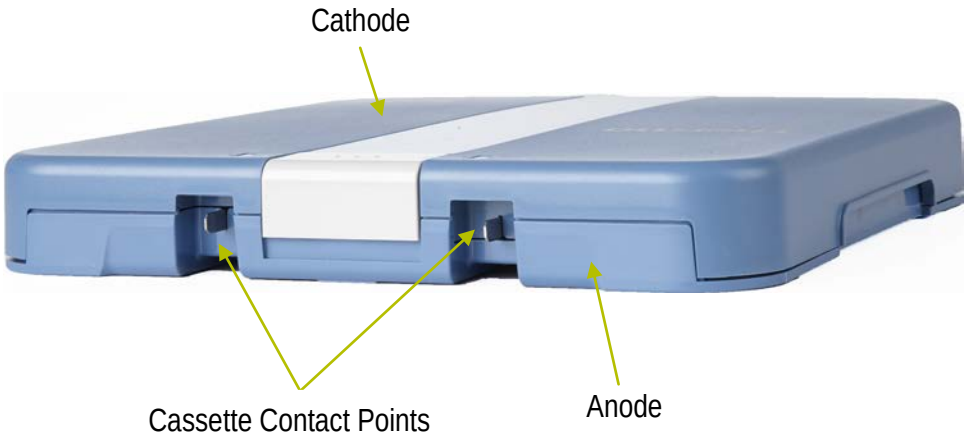


Figure 2.2. Cassette view detailing the upper cathode, the lower anode and the electrical contacts that insert into the control unit contacts.

Figure 2.3. Contol unit with cassette inserted.

Figure 2.4. Rear view of control unit with cassette inserted.

Start Up

Turn the power switch located at the bottom rear of the blotter panel to the On position. The power switch provides power to all of the blotter components. The on-board computer boots up, the touchscreen displays the Thermo Scientific logo followed by “Welcome to the Pierce G2 Fast Blotter,” and then automatically moves to the Main Menu. The blotter is now ready for operation.

Shut Down

On the Main Menu screen, touch the Shut Down button. When prompted “Are you sure you want to shut down?” select Yes. When notified that “It is now safe to turn off the device,” turn the power switch to the Off position.

3

SECTION 3

Pre-Programmed Transfer Methods

Fast Blotting: Constant Amps/Surface Area

Fast blotting methods, such as the Pierce G2 Fast Blotter, require a high-current power supply and an optimized high ionic strength transfer buffer, such as Pierce 1-Step Transfer Buffer. By increasing the current, excellent transfer efficiency can be achieved in a much shorter time. Amperage is held at a constant rate based on the surface area of the transfer stack(s) (~22-23mA/cm^2) and voltage is limited to a maximum of 25V.

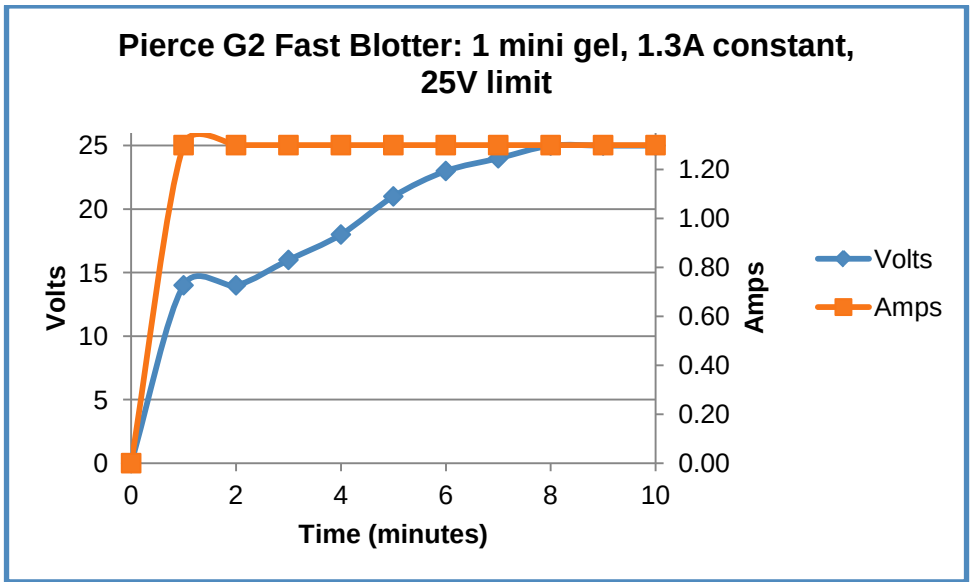


Figure 3.1. Fast blotting protocol (volts vs amps).

Traditional, efficient semi-dry transfer protocols use constant voltage (25V) and low-ionic strength transfer buffer (Towbin) to generate low amperage for an extended length of time (30-60 minutes).

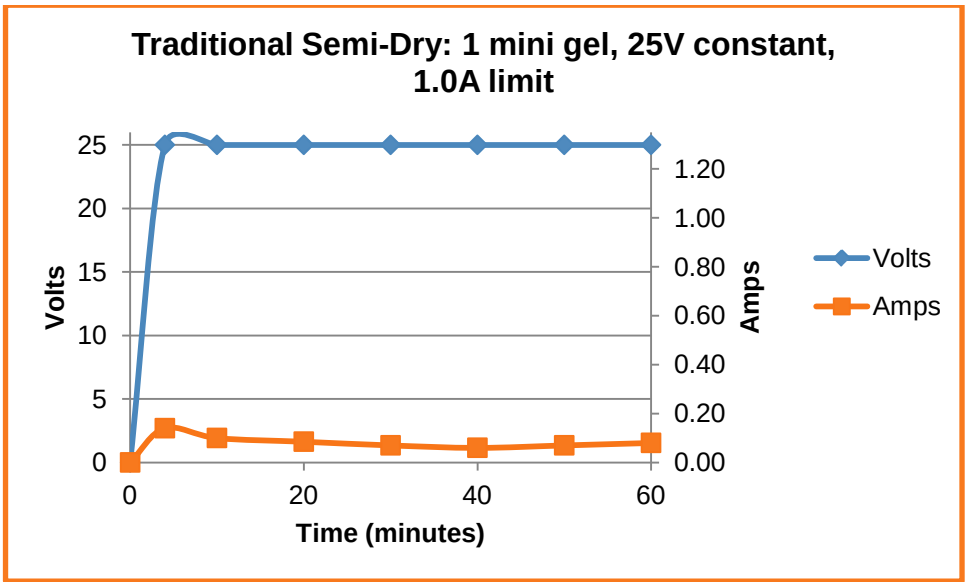


Figure 3.2. Traditional semi-dry blotting protocol (volts vs amps).

Pierce 1-Step Transfer Buffer

Simultaneously transfer one to four mini-sized gels or one to two midi-sized gels in 5-10 minutes. Gels simultaneously transferred must have the same formulation. Mini gels are defined as ~46cm^2 and 60cm^2. Midi gels are defined as ~110cm^2. Note that these dimensions are the surface area of the gel with the gel fingers removed (not of the cassette).

1. Cut four sheets of ~0.83mm thick Western blotting filter paper and one sheet of nitrocellulose or PVDF membrane to the same size as the gel(s).

   Note The transfer stack should contain two sheets of ~0.83mm thick filter paper on the bottom (anode), followed by membrane, gel and two sheets of ~0.83mm thick Western blotting filter paper on top. If 0.83mm thick filter paper is not available, do not exceed 1.8mm total filter paper thickness for the bottom of the stack or 1.8mm total filter paper thickness for the top of the stack. Use the appropriate Thermo Scientific filter papers:
   Product No. 84873, 0.83mm thick, 7.0cm × 8.4cm (mini-size)
   Product No. 84874, 0.83mm thick, 8.0cm × 13.5cm (midi-size)
   Product No. 88600, 0.83mm thick, 8.0cm × 10.5cm

2. Equilibrate filter paper and membrane in Pierce 1-Step Transfer Buffer for a minimum of 5 minutes. Use sufficient buffer to completely cover the filter paper and membrane.

   Note PVDF membrane must be wetted with methanol or ethanol before equilibration in Pierce 1-Step Transfer Buffer. After electrophoresis, remove gel from cassette(s) and briefly place in a tray containing deionized water or transfer buffer. This will ensure even wetting, facilitate proper gel placement and improve contact with the membrane.

3. Assemble stack centered on the bottom half of the cassette (anode) as depicted in Figure 3.3. Use a blot roller to remove any trapped air bubbles.

   Note Removal of trapped air bubbles is essential for high-quality transfer. Two firm passes with the included blot roller is typically sufficient.

   Note If assembling more than one stack on the anode surface, evenly space and center the sandwiches so the top cathode surface applies even pressure to the surface areas of the stack(s). Ensure there is a 1cm space around all stack edges to allow any gases to escape during transfer.

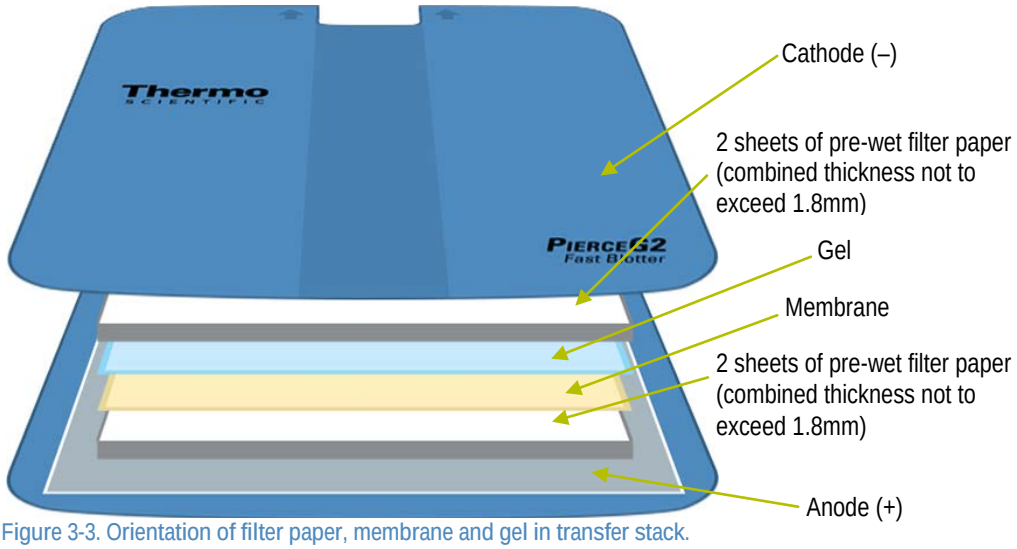
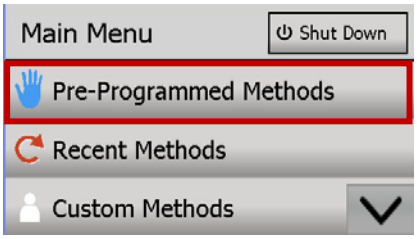


Figure 3-3. Orientation of filter paper, membrane and gel in transfer stack.

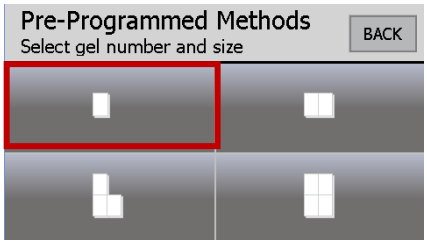
4. Gently press down top of cassette (cathode) to lock into place.

5. Slide cassette into the control unit.

6. Select **Pre-Programmed Methods** in the **Main Menu**.



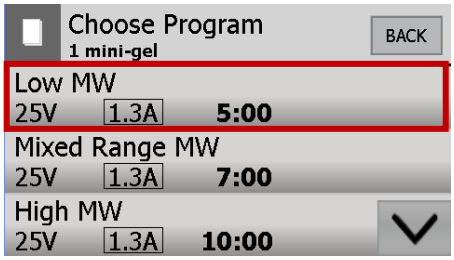
7. Using the touchpad, select the number of gels and gel size (mini or midi) you wish to transfer:



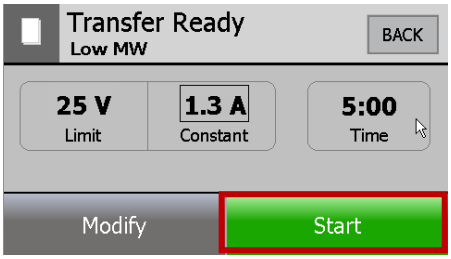
8. Choose the appropriate program to run (constant parameter in box):

a.	Low MW (< 25kDa)	25V	Amps	5:00 min
b.	Mixed Range MW(25-150kDa)	25V	Amps	7:00 min
c.	High MW (> 150kDa)	25V	Amps	10:00 min
d.	Std Semi-Dry	25V	1.0A	60:00 min
e.	1.5mm thick gels or unknown size gels	25V	Amps	10:00 min

Note For fast-blotting programs (a, b, c and e), use Pierce 1-Step Transfer Buffer. Transfer time may be increased to 12 minutes for extremely high molecular-weight proteins or for slow-transferring gels. Do not use the Std Semi-Dry transfer program (d) with Pierce 1-Step Transfer Buffer.



9. Select the Start button to begin transfer.



10. Upon transfer completion, remove the transfer stack from the cassette(s) and thoroughly rinse the top and bottom section of the cassette.

Note Build-up of buffer salts will reduce cassette function and prevent the cassette from properly opening and closing. Rinse cassette(s) after every use.

WARNING After running at high current, the anode and cathode plates can become hot. Use caution when separating the gels and stacks from the plates. When continuously processing multiple samples at ~5A for a maximum of 2 hours, allow the cassette to cool for 30 minutes or use multiple cassettes to avoid excessive cassette heating.

Traditional Semi-Dry Transfer Method

Simultaneously transfer one to four mini-sized gels or one to two midi-sized gels in 45-60 minutes. Gels simultaneously transferred must have the same formulation.

1. Equilibrate gel(s) for 15 minutes in Towbin transfer buffer (25mM Tris, 192mM glycine, 20% methanol).

2. Cut two extra thick (~2.48mm thick) sheets of Western blotting filter paper and one sheet of nitrocellulose or PVDF membrane to the same size as gel(s).

   Note The transfer stack should contain one sheet of ~2.48mm thick filter paper on the bottom (anode), followed by membrane, gel and one sheet of ~2.48mm thick Western blotting paper on top. Do not exceed 2.48mm total filter paper thickness for the bottom of the stack or 2.48mm total filter paper thickness for the top of the stack. Use the appropriate Thermo Scientific filter paper:
   Product No. 88605, 2.48mm thick, 7.0cm × 8.4cm (mini-size)
   Product No. 88610, 2.48mm thick, 8.5cm × 9.0cm
   Product No. 88615, 2.48mm thick, 8.0cm × 13.5cm (midi-size)
   Product No. 88620, 2.48mm thick, 20.0cm × 20.0cm

3. Equilibrate filter paper and membrane in Towbin transfer buffer for at least 5 minutes.

   Note PVDF membrane must be wetted with methanol or ethanol before equilibration in Towbin transfer buffer.

   Note Filter paper and membrane stacks can be prepared ahead (few hours to overnight) and stored at 4°C.

4. Assemble stack on anode as depicted in Figure 3.4. Center stack(s) on anode to ensure even pressure. Use blot roller to remove any trapped air bubbles.

   Note Removal of trapped air bubbles is essential for high-quality transfer. Two firm passes with the included blot roller is typically sufficient.

   Note If assembling more than one stack on the anode surface, evenly space and center the sandwiches so the top cathode surface applies even pressure to the surface areas of the stack(s). Ensure there is a 1cm space around all stack edges to allow any gases to escape during transfer.

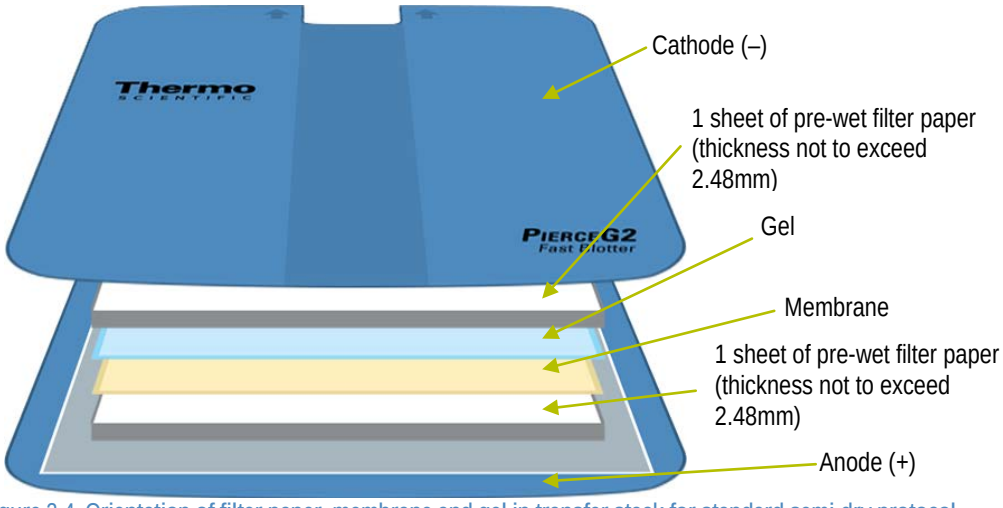
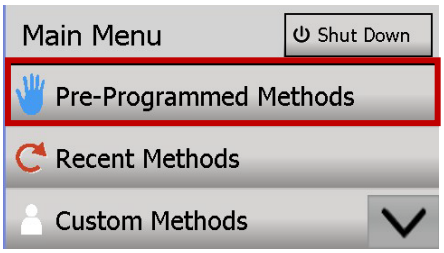


Figure 3-4. Orientation of filter paper, membrane and gel in transfer stack for standard semi-dry protocol.

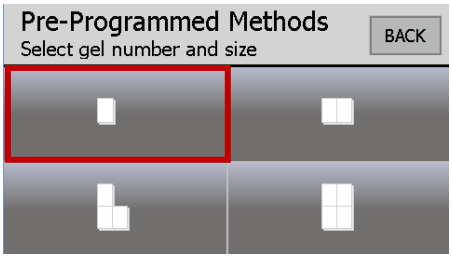
5. Gently press down top of cassette (cathode) to lock into place. Slide cassette into the control unit.

6. Select **Pre-Programmed Methods** in the **Main Menu**.



7. Select the number of gels and gel size (mini or midi) you wish to transfer:

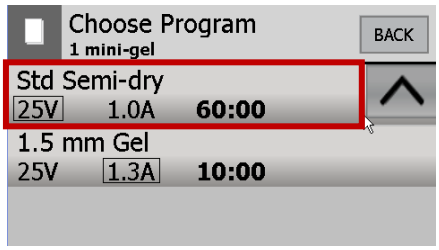
   a. 1 mini-sized gel — 25V
   b. 2 mini-sized gels or 1 midi-sized gel — 25V
   c. 3 mini-sized gels — 25V
   d. 4 mini-sized gels or 2 midi-sized gels — 25V



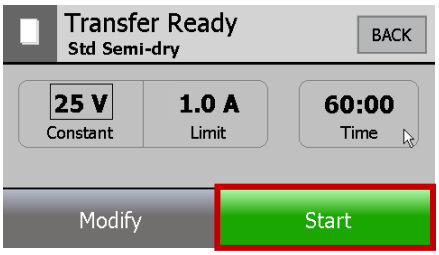
8. Choose program **Std Semi-dry** (constant parameter in box):

a.	Low MW	25V	Amps	5:00 min
b.	Mixed Range MW	25V	Amps	7:00 min
c.	High MW	25V	Amps	10:00 min
d.	Std Semi-dry	25V	1.0A	60:00 min
e.	1.5 mm thick gels or unknown size gels	25V	Amps	10:00 min

Note For Std Semi-dry program (d), use Towbin transfer buffer or other conventional transfer buffer.



9. Select the **Start** button to begin transfer.



After running at high current, the anode and cathode plates can become hot. Use caution when separating the gels and stacks from the plates.

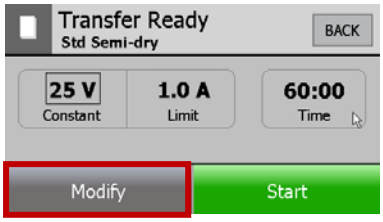
4

SECTION 4

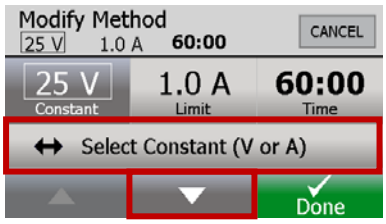
Custom Methods

Modifying Pre-Programmed Methods

Pre-programmed methods can be modified before **Start** by selecting the **Modify** button.

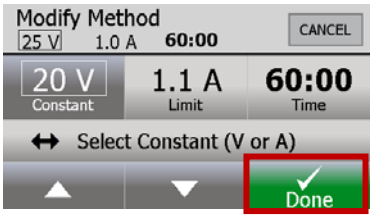


1. Pressing the **Select Constant (V or A)** button will toggle the constant variable parameter from amps to volts or volts to amps.



2. Highlight the variable for change and press the **Up** or **Down** arrow to raise or lower the selected variable's value.

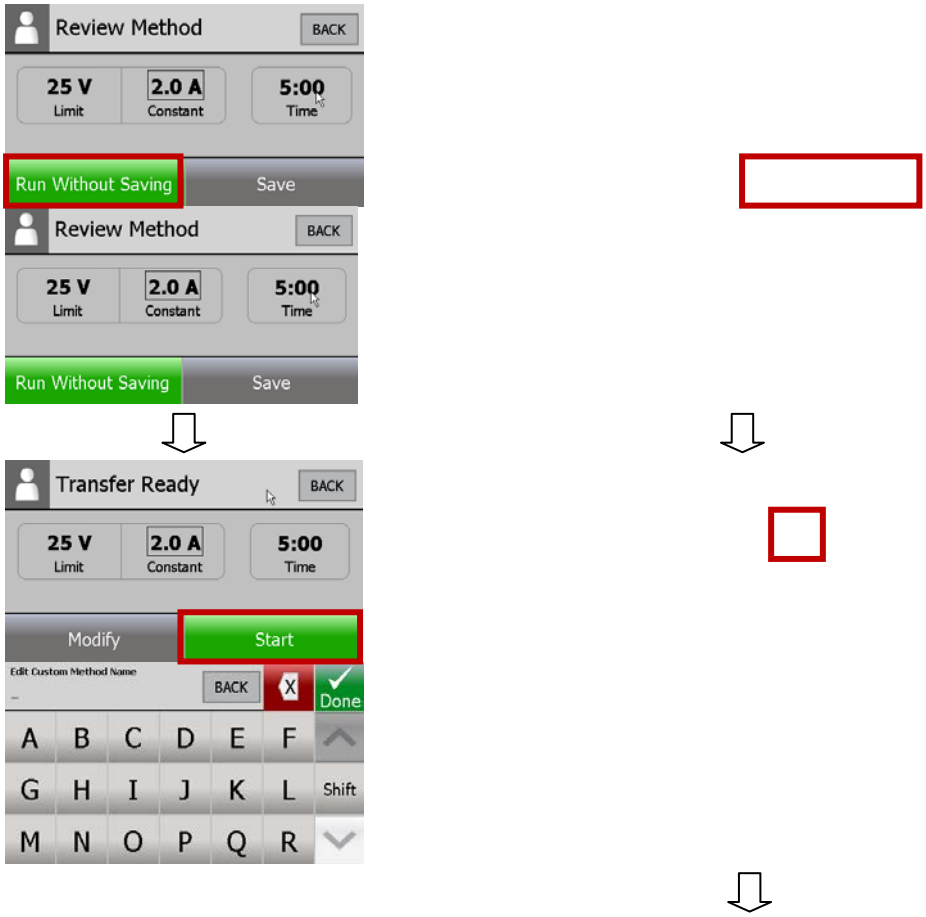
3. After the program is modified, press the **Done** button.

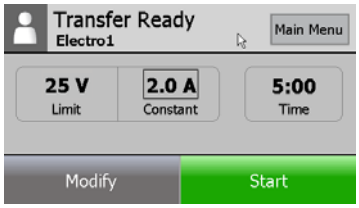


4. The "Review Method" screen is now displayed. Press **Run Without Saving** or **Save**.

   a. **Run Without Saving** will prompt the "Transfer Ready Screen." Press **Start** to begin the modified method.

   b. If you wish to save the modified method, press **Save** and use the alphanumeric key pad to enter up to 15 characters to identify the new custom method. Press **Done** and then **Start** to begin the method. The custom method will be saved in **Custom Methods** in the **Main Menu**.





Creating a New Custom Method

Custom methods are typically used when transferring non-standard gel sizes (i.e., not mini- or midi-sized). For rapid transfer protocols using Pierce 1-Step Transfer Buffer, measure the total surface area of the gel(s) that you are transferring and multiply by 23mA/cm^2. For example, if transferring a 12cm × 15cm non-standard gel (measuring the gel and not the cassette), then use you would use the following formula:

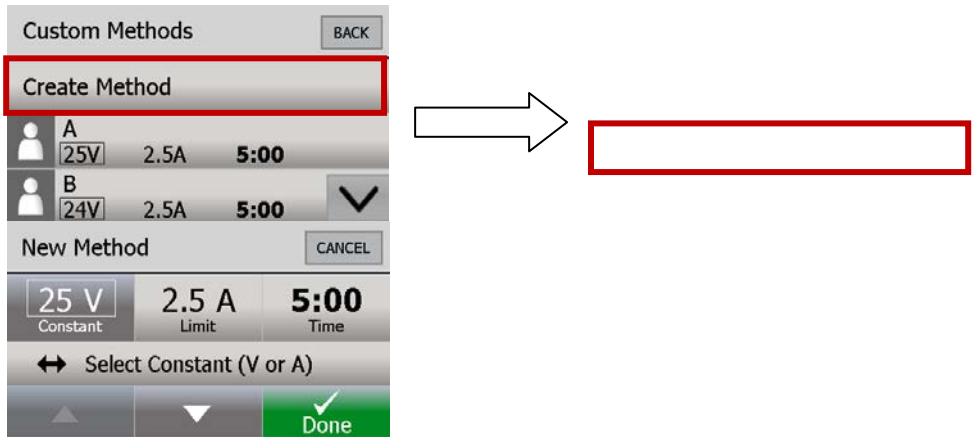
(12cm × 15cm × 23mA/cm^2) ÷ 1000mA/A = 4.1A (constant)

The voltage limit will be set at 25V. The amperage will be constant and set at 4.1A. The time will be set at 7 minutes for mid-range molecular weight proteins (25-150kDa).

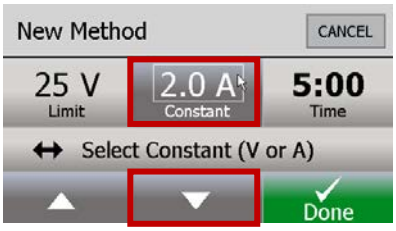
Note Five amps is the maximum current setting allowed. If the gel area exceeds 220cm^2, set the amperage to 5A and compensate by increasing the transfer time by 1-2 minutes. For standard semi-dry protocols using Towbin buffer, voltage is held constant at 25V, amperage is limited to 1.0A and time is set to 30 minutes to 1 hour. Standard semi-dry programming does not take surface area into consideration.

1. To create a custom method, press the Custom Method button on the Main Menu.

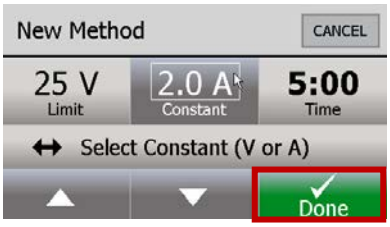
2. Select the Create Method button and press Select Constant (V or A) to toggle the constant variable parameter from amps to volts or volts to amps.



3. Highlight the variable for change and press the Up or Down arrow to raise or lower the selected variable's value.



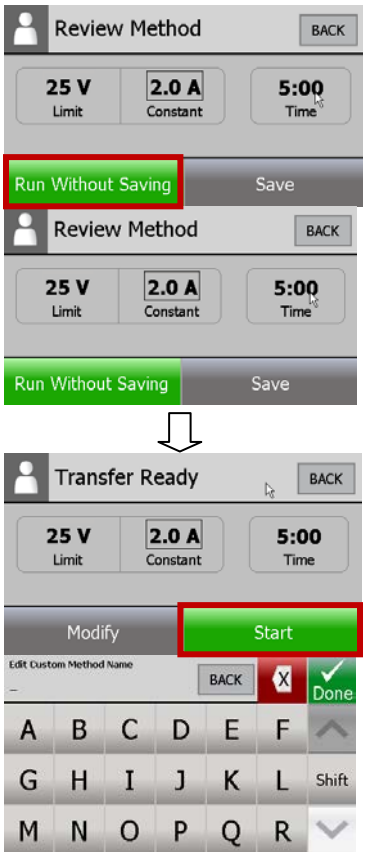
4. After the program is modified, press the Done button.



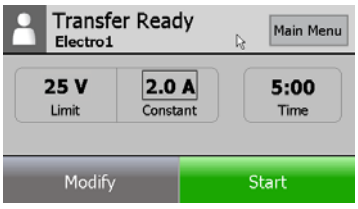
5. The Review Method screen is now displayed. Select Run Without Saving or Save.

   a. Run Without Saving will prompt the Transfer Ready screen. Press Start to begin the modified method.

   b. If you wish to save the modified method, press Save and use the alphanumeric keypad to enter up to 15 characters to identify the new custom method. Press Done and then Start to begin the method. The custom method will be saved in Custom Methods in the Main Menu.

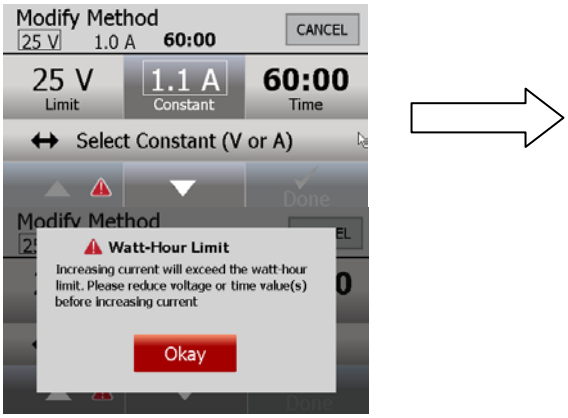

Review Method
BACK
25 V
Limit
2.0 A
Constant
5:00
Time
Run Without Saving
Save
Review Method
BACK
25 V
Limit
2.0 A
Constant
5:00
Time
Run Without Saving
Save
Transfer Ready
BACK
25 V
Limit
2.0 A
Constant
5:00
Time
Modify
Start
Edit Custom Method Name
BACK
X
Done
A B C D E F
G H I J K L
Shift
M N O P Q R

Edit Custom Method Name
Electro1_
BACK
X
Done
s t u v w x
y z 1 2 3 4
Shift
5 6 7 8 9 0


Transfer Ready
Electro1
Main Menu
25 V
Limit
2.0 A
Constant
5:00
Time
Modify
Start

Recommended Maximum Watts/Hour Limit

To prevent damage to the cassette or control unit, custom methods are limited to 25 watts/hour (when modifying pre-programmed methods and/or creating a new method). Any voltage or amperage adjustments that result in exceeding the maximum 25 watts/hour will bring up an alert triangle on the Up arrow key. Continuing past this value will eventually result in a Watt-Hour Limit warning window.



If the Watt-Hour Limit warning window appears, select the Okay button to return to the previous screen and decrease appropriate number to return to a watt-hour value less than 25 watts/hour.

1. Pierce 1-Step Transfer Buffer is intended to rapidly transfer protein from gel to membrane using a high transfer current (based on transfer area) and a short time (5-10 minutes).

    a. Do not exceed 12-minute transfer times with rapid transfer protocols.

    b. Do not exceed 22-23mA/cm^2 (current/surface area) with rapid transfer protocols.

2. Conventional transfer buffers such as Towbin buffer (25mM Tris, 192mM glycine, 20% methanol) are low ionic strength buffers and transfer time ranges from 45-60 minutes. Current normally spikes and then quickly drops to a very low level, while voltage reaches its maximum (25 volts).

5

SECTION 5 Maintenance

Cleaning the Blotter LCD Touchscreen

IMPORTANT Use of ammonia-based cleaners to clean the touchscreen is NOT recommended. Do not spray cleaner directly onto the LCD screen.

IMPORTANT Do not press hard while cleaning the screen. Do not leave excess cleaning solution on the LCD screen as this may cause long-term damage.

1. Use a pre-moistened LCD screen-cleaning tissue to clean the surface of the touchscreen.

Cleaning the Blotter Housing and the Cassette

IMPORTANT When using cleaning products with the blotter, consult the appropriate material safety data sheet (MSDS) to confirm compatibility and proper usage of the cleaning product.

1. Spray a soft cloth or paper towel with cleaning solution (e.g., general-purpose cleaner containing water and mild detergent, Isopropanol, or 70% ethanol). Avoid abrasives and organic solvents.
2. Wipe the exterior surfaces of the control unit and cassette. Use caution to avoid touching the LCD touchscreen with anything other than a screen-cleaning wipe.
3. Ensure that the cooling fan vent is free of dust and debris.

Cleaning the Anode and Cathode Plates

IMPORTANT Always wear gloves when touching the anode and/or cathode.

Note Pierce 1-Step Transfer Buffer is a highly concentrated salt solution. Build-up of buffer salts will reduce cassette function and prevent the cassette from properly opening and closing.

1. After each use, thoroughly rinse the anode and cathode under warm water while removing any sticky salt residue with a gloved hand. If the cathode has excessive salt build-up, use a mild acetic acid solution (< 5%) to remove the residue.

2. Rinse with deionized water and stand parts in a rack to dry.

Troubleshooting

Problem	Possible Cause	Solution
Cassette is difficult to open	Salt deposited on moving parts inside cassette	Rinse the cassette top and bottom under warm water while removing any sticky salt residue with a gloved hand. Briefly rinse with deionized water and place in a rack to dry. For more thorough cleaning, immerse the unassembled cassette in warm water, use a gloved hand or clean sponge to remove any sticky salt residue. Rinse with deionized water and place perpendicular in a rack to dry. NOTE: Failure to keep cassette top (cathode) and bottom (anode) clean will result in the sticking of moving parts and lead to poor transfer efficiency
Inconsistent transfer	Membrane or filter paper was insufficiently equilibrated in Pierce 1-Step Transfer Buffer	Equilibrate membrane and filter paper in Pierce 1-Step Transfer Buffer before transfer for a minimum of 5 minutes. Use a sufficient amount of buffer for the equilibration step
	Insufficient transfer time	Increase transfer time from 7-10 minutes to 10-12 minutes
	PVDF membrane was not pre-wetted with methanol or ethanol	Wet PVDF membrane with methanol or ethanol and equilibrate for 10-15 minutes in Pierce 1-Step Transfer Buffer before transfer
	Air bubbles trapped between gel and membrane	When assembling transfer stack, use a roller or pipette to remove any air bubbles between the gel and the membrane
	Filter paper used in the fast transfer exceeded 1.8mm	Use filter paper < 1.8mm thick
Inefficient transfer of low molecular-weight proteins to PVDF	Inefficient binding of some low molecular-weight proteins (< 25kDa) to PVDF membrane	Combine ethanol and Pierce 1-Step Transfer Buffer in a 15:85 ratio before equilibrating filter paper and membrane (e.g., 15mL of ethanol with 85mL of Pierce 1-Step Transfer Buffer)

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